Recent trends in the role of micro encapsulation in the development of bio-functional foods

K. Kailasapathy, H. Madziva, K. Anjani, S. Seneweera and M. Phillips,
University of Western Sydney, Richmond, Australia (k.kailasapathy@uws.edu.au)

Introduction

Bio-functional foods offer physiological health benefits and disease prevention over and above their nutritional contribution. Micro encapsulation has become the recent tool used for protecting and delivering bio-actives in the development of bio-functional foods. Probiotic foods are by far the largest functional food market. They provide several health benefits including immuno stimulation. Viability, physiological and metabolic activity of these bio actives in a food product at the point of sale are important consideration for their efficacy, as they have to survive during shelf life of a food, transit through high acidic and alkaline conditions in the gastro-intestinal tract (git). Folic acid fortification in foods is mandatory in several countries due to its role in preventing neural tube defects, protective role against coronary heart disease by reducing the levels of homo cysteine in the blood and preventing certain forms of cancer. Novel biotechnological processes such as accelerating cheese ripening and production of therapeutic bioactive peptides that provides, for example, appetite control in the reduction of obesity are being developed. Micro encapsulation is an inclusion technique for entrapping a bio-functional nutrient or bio-active compound such as probiotic bacteria, folic acid and protease enzymes into a polymeric (gelled) matrix that may be coated by one or more semi-permeable polymers, by virtue of which the encapsulated substance become more stable than the free one. The aim of our investigation was to study the survival of micro encapsulated bacteria in fermented dairy products and their release in the git and immune stimulating effects; stability, release and therapeutic effects of micro encapsulated folates and the controlled release of flavour enhancing enzymes to accelerate cheese ripening and to study proteolysis during cheese ripening using reverse phase HPLC.

Materials and methods

Starter Cultures, folates and enzymes: The probiotic cultures were obtaibed from the CSIRO starter collection, Australia. Commercial yoghurt cheese starter cultures were obtained from DSM Food Specialties, Australia. *L. casei Shirota* was isolated from Yakult® dairy drink. Folic acid was obtained from Roche Pty Ltd, and Flavourzyme, a fungal protease-peptidase complex was obtained from Novozymes Pty Ltd, Australia. **Microencapsulation:** Encapsulation of probiotic bacteria was performed by the method of Chandramouli *et al*. (2004)[1]. Folic acid was encapsulated using an alginate-pectin polymer mixture as described in Madziva *et al*. (2004)[2]. Flavourzyme was encapsulated with alginate and coated with chitosan as reported in Kavya *et al.* (2006)[3]. **Yoghurt Production**: Several batches of yoghurts were made with probiotic cultures incorporated into yoghurt mix in different states: free and encapsulated. The probiotic cultures were incorporated at the same time as the yoghurt culture was added and ABT yoghurts (*L. acidophilus, B. infantis* and *S. thermophilus)* were made. Yoghurt was prepared as described in Iyer and Kailasapathy (2005)[4].
Cheddar Cheese Production: Several batches of Cheddar cheese were made with *L. acidophilus* L10 and *Bifidobacterium* B94 added as free bacteria or encapsulated. Cheddar cheese was prepared as described in Godward and Kailasapathy (2003)[5] with the probiotics added to the milk at the same time as the starter cultures. Microencapsulated bacteria, flavourzyme and folates were added to the cheese milk just before the addition of rennet. **Enumeration of Probiotic Bacteria**: The media used for *L. acidophilus* and *Bifidobacterium* spp were MRS-salicin and MRS-LP respectively. For cheese a medium based on Reinforced Clostridium Agar (RCA) with the addition of aniline blue (0.3g L^{-1}) and dicloxacillin (0.0002% w/v; and filter-sterilized) (RCAAD) was used for *Bifidobacteria* and RCA with bromocresol green (0.004%) and clindamycin 0.1μg/ml (RCABC)

was used for *L. acidophilus*. **Folate determination**: Folate was measured by using the TECRA enzyme-protein-binding assay with a plate reader (Madziva et al., 2004)[4]. **Flavourzyme assay:** Flavourzyme protease-peptidase enzyme was assayed using L-leucine-p-nitoranilide as the substrate and the change in absorbance was measured in a plate reader. **Controlled Release of bacteria.** Equal numbers of free and encapsulated *L. casei Shirota* were incubated at 37ºC in gut contents taken from different areas of porcine gastrointestinal tract and samples taken at selected times and assessed for the probiotic isolate. **Immunomodulation.** Mice were fed 50μg of encapsulated or free *L. casei Shirota* culture (each≈1.7 x 10^8 CFU) for 14 days and then animals were sacrificed and tissues sampled for the cytokine IFN-γ using ELISA kits. **HPLC:** Water soluble and insoluble fractions of the ripening cheese were separately analysed by RP-HPLC and SDS-PAGE. Blood homocysteine levels were measured by RP-HPLC using fluorescent thiol derivatives.

Results and discussion

The results showed that there were losses in the cell numbers of both free and encapsulated probiotic bacteria in set yoghurt over a period of 7 weeks at 5C (Fig.1). There was approximately 4 and 3 log cycle loss in number of cells of both free *L. acidophilus* and *B. lactis* respectively. The encapsulated bacteria, however, showed only 2 log decreases in cell numbers. The low viability in yoghurt is mainly attributed to the lower pH in yoghurt and further reduction of pH in yoghurt during post-acidification. The results showed that encapsulation helps to protect cells from acidity of yoghurt. In Cheddar cheese stored over six-months, there was a consistent decrease in cell counts over the storage period, however the free bacteria decreased at a greater rate with both *L. acidophilus* L10 and *Bifidobacterium* B94 falling to between 10^5 and 10^6CFU/g after 6 months. In contrast the encapsulated cultures decreased much more slowly remaining near or above 10^{-7} over this time period (Fig 2).

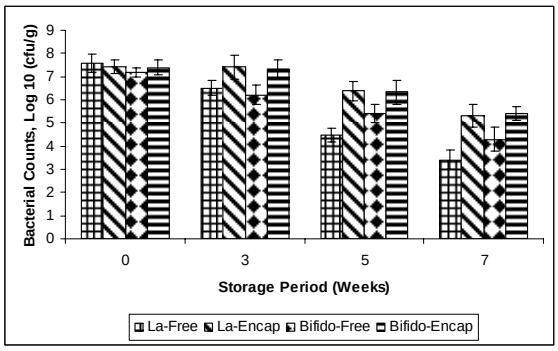


Fig.1 Survival of free and encapsulated *L. acidophilus and Bifidobacterium lactis incorporated into yoghurt during storage*[6]

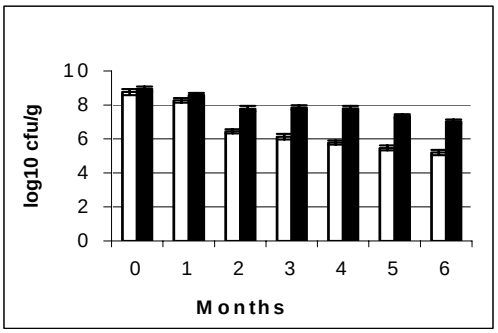


Fig.2. Counts of *Bifidobacterium* in cheddar cheese (Free □ Encapsulated ■)

These results suggest that encapsulation may be a valuable method for producing cheeses that retain sufficient viable bacteria to be classified as probiotic using the suggested standards.

An important feature of microencapsulation is that it requires that the entrapped bacteria be released in an appropriate area of the git. When porcine git contents were inoculated with *L. casei Shirota* and the release of this organism from micro encapsulated beads was measured, it was found that the organisms were in highest numbers and most rapidly released in the ileal contents and in equal numbers, but more slowly in colonic digesta (Fig.3). In comparison there was little or no release in other sites suggesting that the beads would release bacteria in the small bowel, with most released by the time the digesta had reached the colon. When mice were inoculated with *L. casei Shirota* and the ability of the cultures to stimulate the production of the cytokine IFN-γ it was found that the encapsulated cells performed as well or better than free cells (Fig.4).

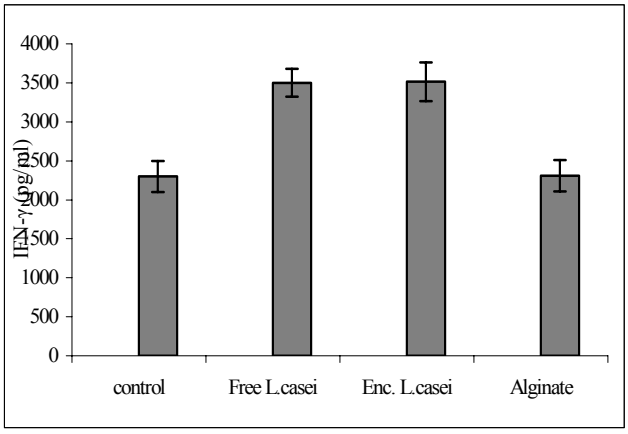


Fig 4

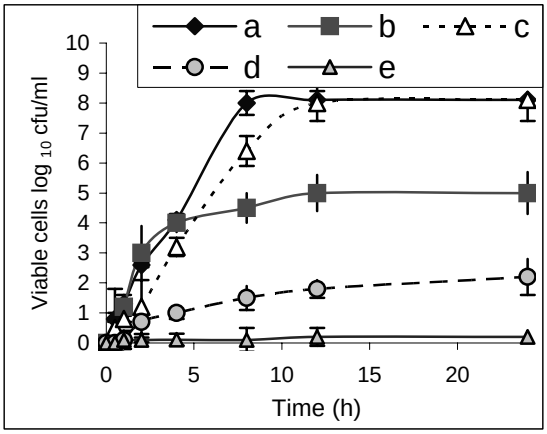


Fig 3

Fig.3. Release of encapsulated bacteria in porcine intestinal contents. a) *Lactobacillus casei Shirota*; standard deviation of mean (n=3). Symbols: a-Ileum, b- Jejunum, c-Colon, d- Duodenum, e-stomach[7]

Fig.4. Effect of free and chitosan-coated alginate-starch encapsulated *Lactobacillus casei Shirota* on mice IFN-γ production. The error bars represent group mean ± SD (n = 7 mice)

When different alginate polymer concentrations were used for micro encapsulating Flavourzyme and hardening for 10 min in 0.1M calcium chloride solution containing 0.1M (w/v) chitosan, there was no increase in the encapsulation efficiency of Flavourzyme on increasing the alginate concentration beyond 1.6% (w/v) (Fig.5). Coating of the hydro-gel capsules with polymers has been shown to reduce the porosity of the capsules and enhance the retention of the load. However, when the hydro-gel capsules were coated with alginate and poly-L-lysine, there was no improvement in the enzyme encapsulation efficiency. These results showed that unlike previously published encapsulation of proteases in liposomes, the encapsulation efficiency could be increased significantly by using hydro-gels made with alginate and chitosan polymers, hence, a significantly better method of delivery of proteases enzymes into the cheese matrix for accelerated cheese ripening.

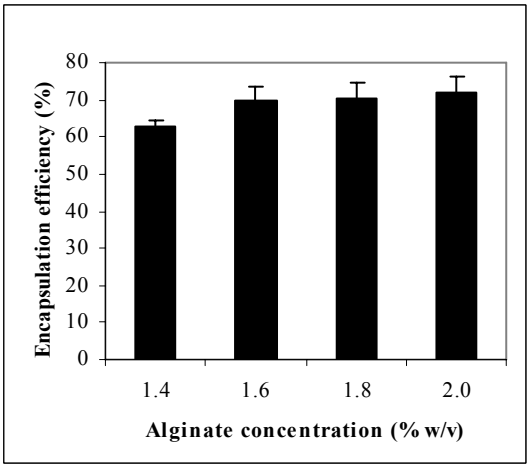


Fig. 5. Effect of alginate on EE of flavourzyme[3]

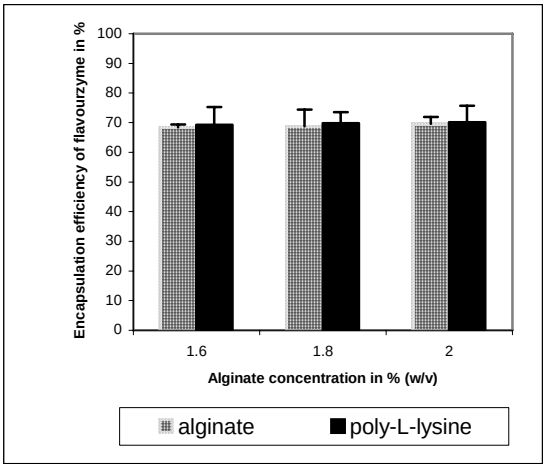
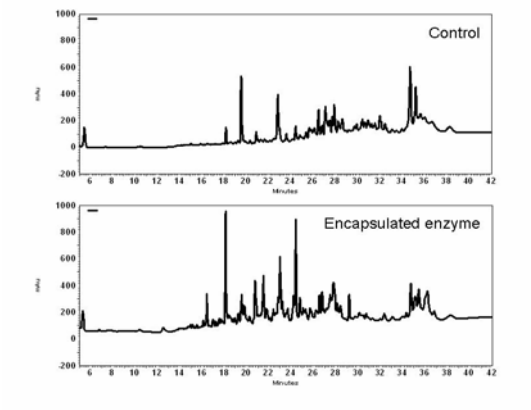


Fig. 6. Effect of 0.15% alginate and 0.05% ploy-L Lysine on EE of flavourzyme[3]

It was expected that incorporating microencapsulated alginate-chitosan hydro-gel capsules containing Flavourzyme at a rate of 1.38 units per g of protein into the cheese matrix, would accelerate cheese ripening (Fig.7; Fig.8).



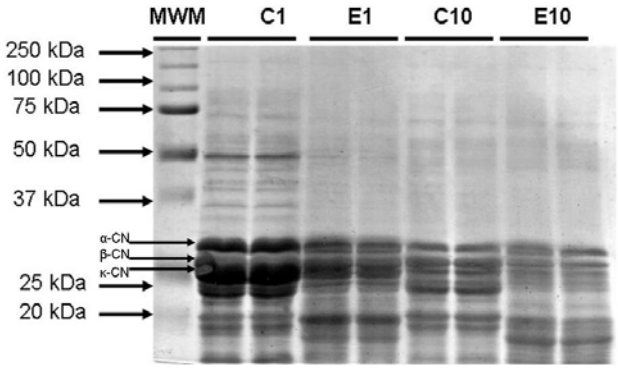


Fig. 7. HPLC of cheese peptide profile

Fig. 8. SDS-PAGE profile cheese peptides

As shown in the HPLC chromatogram (Fig.7) and the SDS-PAGE gel electrophoretogram (Fig.8), compared to the control cheese, encapsulated Flavourzyme treatment significantly enhanced the cheese ripening as seen by the increased amount of peptides in the cheese and the reduction of the casein quantity as cheese ripening proceeds to 70 days. When folic acid (encapsulated with alginate-pectin and hardened in 0.1M calcium chloride solution) were incorporated into cheese, encapsulated folic acid showed greater stability in Cheddar cheese over a 3 month ripening period compared to free folic acid (Honest et al., 2006)[8]. When hyper-homocysteinemia was induced in mice, free folic acid induced greater levels of hyper-homocysteinaemia compared to encapsulated folic acid. In all treatments, encapsulated folic acid diet showed cumulatively lower homocysteine levels than free folic acid ($p<0.05$).

Conclusions

The study showed that microencapsulation would be an effective tool in the development of bio-functional foods and processes.

References

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